Characterisation of nasal methicillin-resistant *Staphylococcus aureus* isolated from international human and veterinary surgeons

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Abstract

Nasal colonization with methicillin-resistant *Staphylococcus aureus* is poorly described for surgeons, despite the increased exposure to nosocomial pathogens and at-risk patients. This study investigated the molecular epidemiology and antimicrobial resistance of 26 MRSA isolates cultured from the nares of an international cross-sectional study of 1,166 human and 60 veterinary surgeons. All isolates were subjected to *agr*-, *spa*- and MLST typing and the presence of 22 virulence factors were screened for by PCR. Additionally, biofilm-forming ability, haemolytic activity, staphyloxanthin production and antibiotic resistance were determined. The genome of a rifampicin resistant MRSA was sequenced. Approximately half of the isolates belonged to well-described clonal lineages, ST1, ST5, ST8, ST45 and ST59, that have been previously associated with severe infections and increased patient mortality. Two of the 3 veterinarian MRSA belonged to epidemic livestock-associated MRSA clonal lineages (ST398 and ST8) previously associated with high transmission potential between animals and humans. The isolates did not display any consistent virulence gene pattern, and 35% of the isolates carried at least one of: the Panton-Valentine leukocidin *lukFS-PV*; the exfoliative toxin *eta*; or the toxic shock syndrome *tst* genes. Resistance to rifampicin was detected in one veterinarian isolate, and was found to be due to 3 mutations in the *rpoB* gene. Surgeons occupy a critical position in the healthcare profession due to their close contact with patients. In this study, surgeons were found to be colonized with MRSA at low rates that are similar to the general population, and the colonising strains were often common clonal lineages.

Introduction

Staphylococcus aureus colonizes between 26-35% of healthy humans, with the moist squamous epithelium of the anterior nares considered the primary reservoir (1-4). Due to its importance as a pathogen responsible for a wide range of difficult-to-treat infections, colonization with methicillin-resistant *S. aureus* (MRSA) has been investigated extensively in patients, but to a

lesser extent in their primary care-givers, including health care workers (HCWs) such as orthopaedic surgeons. Studies have consistently shown that average MRSA colonization rates are between 1-3% in the general population (5, 6), with increased rates shown for certain groups such as the elderly in assisted living facilities (6) or long-term in-patients (2), and those with immunity defects such as HIV, chronic granulomatous disease (CGD), Job's syndrome, Chediak-Higashi syndrome, and Wiskott-Aldrich syndrome (7, 8).

Recently, the MRSA colonization rate in an international cohort of human and veterinary orthopaedic surgeons was investigated (9). From a total cohort of 1,166 human surgeons, the MRSA colonization rate was 2% (23/1,166) and was 5% (3/60) amongst the veterinary surgeons (9). What remains undocumented within this study is the molecular epidemiology of these MRSA isolates. Previous studies have identified that distinct clonal lineages belonging to different sequence types (STs) or clonal complexes (CCs) have emerged and spread across the world (10-12). For example, USA300 isolates (ST8) have been documented across the US and beyond, (11). Interestingly, livestock-associated (LA)-MRSA such as ST398 have been found on humans living and working on farms (13-15). It has also been shown that veterinary surgeons are at increased risk of carrying the same epidemic MRSA isolates as the animals (13-15). This clearly demonstrates the possibility of transmission between animal and human carrier and *vice versa*.

We hypothesize that the MRSA nasal isolates collected from a cross-sectional study from human and veterinary surgeons will belong to well-known MRSA lineages. Therefore the aim of this study was to phenotypically and genotypically characterise the 23 MRSA nasal isolates from human surgeons as well as 3 MRSA isolates from veterinary surgeons, and determine the clonal relationship of these nasal isolates to known globally disseminated MRSA lineages.

Materials and Methods

Methicillin-resistant *Staphylococcus aureus* (MRSA) collection

MRSA isolates were obtained from a previously described study (9). Twenty-three MRSA isolates were recovered from the nares of 1,166 human orthopaedic surgeons sampled on an anonymous basis during an educational course in Switzerland in 2013. Furthermore, 3 additional MRSA isolates were collected from 60 veterinary orthopaedic surgeons not described in the previously study (9) but were included in the present study. The study was approved by the "Ethik-Kommission der Bayerischen Landesärztekammer", Germany (Approval number 13090). Beside a nasal swab all participants gave information on basic demographic and professional questions such as location of place of work and birth (country and region). Bacterial isolation and identification was performed as described previously (9). After identification a single colony was taken and resuspended in 1 ml Tryptone Soy broth (TSB, Sigma Aldrich, Buchs, Switzerland) containing 20% vol/vol glycerol and stored at -80°C. Isolates were re-grown either on Tryptone Soy Agar (TSA, Sigma Aldrich, Buchs, Switzerland) plates or in TSB media for phenotypic characterization or for genomic DNA isolation. The 23 MRSA human orthopaedic surgeon isolates and their antimicrobial resistances were partially described by Morgenstern et al. 2016 (9). A more detailed profile of each individual isolate was analysed in this study.

Phenotypic characterisation

Antibiotic susceptibility testing

Antibiotic susceptibility to 28 antibiotics (amikacin, ampicillin-sulbactam, cefotaxim, cefoxitin, cefuroxime, ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid, gentamicin, levofloxacin, linezolid, mezlocillin, moxifloxacin, netilmicin, ofloxacin, oxacillin, penicillin, piperacillin, rifampicin, tetracycline, ticarcillin/clavulanate, tigecycline, tobramycin, trimethoprim-sulfamethoxazole and vancomycin) were determined using a Vitek2 machine (bioMérieux Vitek Inc., Hazelwood, MO, USA). Multiple antibiotic resistance was defined according to the definitions of the European Committee of Antimicrobial Susceptibility Testing (EUCAST). Oxacillin resistance was considered definitive for methicillin resistant status.

Cefoxitin disc diffusion test

To confirm isolates as methicillin resistant, a cefoxitin disc diffusion test was performed at Synlab Suisse (Luzern, Switzerland) using a 30 µg disc. Zone sizes were measured and interpreted according to EUCAST interpretative criteria (http://www.eucast.org/clinical_breakpoints).

Mupirocin disc diffusion test

Resistance to mupirocin was tested on all isolates at Synlab Suisse (Luzern, Switzerland) by disc diffusion test using a 200 µg disc. Zone sizes were measured and interpreted according to EUCAST interpretative criteria (http://www.eucast.org/clinical_breakpoints).

Staphyloxanthin production, haemolytic activity and biofilm production

Staphyloxanthin production was tested as described previously (16) by two independent observers. *S. aureus* Newman and USA300 were included as positive controls for strong and *S. aureus* COL and UAMS-1 for weak staphyloxanthin production, respectively (16).

Haemolytic activity of each MRSA isolate was assessed as described previously (16). *S. aureus* reference isolates USA300 and UAMS-1 were used as controls for strong and absent haemolysis activity, respectively (16).

Biofilm formation was assayed as described previously (17, 18). Briefly, overnight cultures grown in Tryptic Soy Broth (TSB, Sigma Aldrich, Buchs, Switzerland) were suspended in fresh TSB containing 1% glucose, to approximately $1x10^6$ CFU/ml, correlating to an optical density of 0.02±0.005 at 600nm as measured with a Multiskan Go microplate reader (Thermo Scientific, Zürich, Switzerland). A total of 200 µl of the bacterial suspension was incubated in flat-bottomed 96-well tissue culture-treated polystyrene microtitre plates (Nuclon, Nunc A/S, Denmark) for 24 h

at 37 °C. Plates were rinsed with phosphate-buffered saline (PBS, Sigma-Aldrich, Buchs, Switzerland) and stained with 150 µl of Gram`s crystal violet solution (Sigma-Aldrich, Buchs, Switzerland). The dye bound to the attached cells was solubilized by addition of 150 µl of 95% ethanol. Optical density was measured as absorbance at 595 nm using the Multiskan Go microplate reader. All isolates were tested in triplicate in three independent experiments. Each microtitre plate also consisted of negative controls (wells without bacterial inoculation). The results were evaluated using the scale described by Stepanovic et al. (17). *S. epidermidis* reference strain RP12 (ATCC 35983) was used as a control for strong biofilm production.

Genotypic characterisation

DNA extraction

Whole-cell (genomic) DNA, used as template for PCR amplification, was prepared from single colonies using the Wizard® 143 Genomic DNA purification kit (Promega, Dübendorf, Switzerland) according to the manufacturer`s protocol.

PCR amplification

Specific primers, corresponding genes and PCR reaction conditions are previously described by Post et al. (Post et al., 2014) and summarized in Table 1. PCR amplification was carried out in a BioRad MyCycler Thermocycler (BioRad, Reinach Switzerland) in a total volume of 12.5 µl containing 10X Green GoTaq® 151 Reaction buffer (Promega, Dübendorf, Switzerland), 5 mM dNTP Mix (Promega, Dübendorf, Switzerland), 50 pmol of each primer, 1 unit of *Taq* DNA polymerase recombinant (Invitrogen, Zug, Switzerland) and 10-50 ng template DNA.

Accessory gene regulator (agr) typing

agr polymorphisms were detected by PCR as described by von Eiff et al. and Post et al. (18, 19) with primers listed in Table 1.

Microbial surface components recognizing adhesive matrix molecules (MSCRAMM) detection

Genes coding for MSCRAMMs were screened for by PCR with primers previously described (18) with primers listed in Table 1.

Staphylococcal enterotoxins and other toxins

Genes coding for staphylococcal enterotoxins (SEA-SEE), Panton-Valentine leukocidin (PVL) and toxic shock syndrome toxin (TSST) were detected by PCR using primers previously described (18) with primers listed in Table 1.

Methicillin resistance gene

The methicillin resistance gene *mecA* was detected using primers previously described (18) and listed in Table 1.

Spa *typing*

The polymorphic repeat region (region x) of the *S. aureus* protein A encoded by the *spa* gene was amplified as described previously (20). The *spa*-type for each isolate was determined as described by Harmsen and colleagues (21) using the online tool Ridom SpaServer (http://spaserver.ridom.de/) with primers listed in Table 1.

Multi-Locus Sequence Typing (MLST)

MLST was performed with published primers following the instructions on http://saureus.mlst.net/.

DNA sequencing and sequence analysis

PCR products were purified for sequencing using the PureLinkTM Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, Zug, Switzerland) following the manufacturer`s protocol. Automated sequencing was performed at Microsynth AG, Balgach, Switzerland on an Applied Biosystems ABI3730xl Sequence Analyser 5.2 using the ABI Big Dye® 182 system V3.1.

Whole genome sequencing of MRSA-3

Genomic DNA of MRSA-3 isolate was extracted using a Qiagen QiAmp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer`s protocol using 1 µg/ml lysostaphin (Sigma-Aldrich, Buchs, Switzerland and 2 µg/ml lysozyme (Sigma-Aldrich, Buchs, Switzerland) to lyse the bacteria. Sequencing was performed using an Illumina MiSeq benchtop sequencer (Illumina, San Diego, CA, USA), and the 100 bp short read paired-end data was assembled using the *de novo* assembly algorithm within *Velvet* software (version 1.2.08) (22). Resulting data was archived in the Staphylococcal Bacterial Isolate Genome Sequence database (BIGSdb) (23). The *S. aureus* MRSA252 reference genome (Genbank accession number BX571856.1) (24) was used as a basis for defining locus designations, and reference sequences. A ribosomal multilocus sequence typing (rMLST) approach was used to investigate the genetic relationship between MRSA-3 and 181 published *S. aureus* isolates from the National Center for Biotechnology Information (NCBI). Orthologs for the 53 genes encoding the bacterial ribosome protein subunits (*rps* genes) were defined in all isolates by comparison to the finished genome of MRSA252. To estimate the genealogies for these alignments, ClonalFrame, a model-based approach to determining microevolution in bacteria was used (25). The consensus tree represents combined data from three independent runs with 75% consensus required for inference of relatedness. Recombination events were defined as sequences with a length of >50 bp with a probability of recombination ≥75% over the length, reaching 95% in at least one site.

The presence of genes associated with the Staphylococcal Cassette Chromosome (SCC) *mec* complex (*mecA*, *mecR*, *ccrA* and *ccrB*), the *S. aureus* chromosomal *orfX* gene located to the right

196 of the SCC*mec* integration site, ACME (arginine catabolic mobile element) locus (*speG*, *aliD* and
197 *arcA*) and the rifampicin resistance gene (*rpoB*) were investigated for MRSA-3 by BLAST (basic
198 local alignment search tool) comparison to reference genome MRSA252 (23, 26, 27). These genes
199 were considered as being present when a BLAST match with a >70% nucleotide sequence identity
200 on ≥50% of sequence length was recorded. Where present, the genes where mapped onto the
201 rMLST tree to examine the significance of association between the clustering on the tree and the
202 presence of a specific gene.
203
204 Statistical analysis
205 The prevalence of MRSA and the corresponding 95% confidence interval (95% CI) were
206 calculated using SAS software (Version 9.2; Cary, NC, USA).
207
208 **Results**
209 Phenotypic characterisation
210 *Antibiotic resistance profile*
211 The 26 MRSA isolates were collected from carriers coming from different regions of Africa
212 (2/59), Asia (14/252), Central America (1/22), Europe (6/673) and South America (3/115) (Table
213 2). The MRSA rate was 2% (23/1,166; 95% CI 1.3;2.9) for human surgeons and 5% (3/60; 95%
214 CI 1.0;13.9) for veterinary surgeons. All MRSA isolates had cefoxitin zone diameters <22 mm,
215 confirming their methicillin resistance status. The *mecA* gene was also detected by PCR in all 26
216 MRSA isolates, which is in concordance with the cefoxitin disc diffusion test and the results
217 provided by the oxacillin test by the Vitek2 system. The antibiotic susceptibility profile of all
218 MRSA isolates is listed in Table 2. Fifty percent (13/26 isolates) of all isolates were resistant to
219 erythromycin and clindamycin of which 6/13 isolates showed also resistance to the 4 quinolones
220 ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin. Furthermore, 30.8% (8/26 isolates) were
221 resistant to the 4 aminoglycosides gentamicin, tobramycin, amikacin and netilmicin. Half of the

aminoglycoside resistant isolates (15.4%) showed also erythromycin and clindamycin resistance. All 26 MRSA isolates were susceptible to mupirocin, the only agent approved for de-contamination of MRSA nasal carriage.

Haemolytic activity, staphyloxanthin production and biofilm production

Forty-two percent of isolates displayed the yellow-orange or yellow colony pigmentation indicative of staphyloxanthin production (Table 3) and 69% of isolates were haemolytic (Table 3). One isolate was a strong biofilm producer, and 21 isolates did not produce a biofilm under the described experimental conditions. The remaining 4 isolates produced an intermediate biofilm.

Genotypic characterisation

Accessory gene regulator (agr) typing

Fifty-four percent of isolates belonged to *agr* type I, followed by 27% *agr* type II and 12% *agr* III (Table 3). Two isolates did not belong to any of the 4 *agr* types.

Microbial surface components recognizing adhesive matrix molecules (MSCRAMM) detection

The virulence genes *hlg*C/B (gamma-hemolysin), *icaA* (intercellular adhesion), *eno* (laminin binding protein), *clfA*/*clfB* (fibrinogen binding protein) and *sdrC* (beta-neurexin binding protein) were present in all isolates (Table 3). Ninety-six percent and 85% of isolates carried the *sdrE* (platelet aggregation) gene and *sdrD* (fibrinogen binding protein) gene, respectively, while 73%, 62%, 39% and 39% of isolates possessed the *fib* (fibrinogen binding protein), *ebpS* (elastin binding protein), *cna* (collagen binding protein) and the *fnbB* (fibrinogen binding protein and elastin binding protein) genes, respectively (Table 3). The *bbp* (bone sialoprotein binding protein) gene was not detected in any of the MRSA isolates.

Staphylococcal enterotoxins and other toxins

Two isolates carried the Panton-Valentine leukocidin (PVL) *lukFS-PV* gene and 4 isolates possessed the *tst* (toxic shock syndrome toxin, TSST) gene; all 6 isolates originating from Asia (Table 3). Half of the isolates carried one of the 5 genes coding for staphylococcal enterotoxins (SE) A, B, C, D and E. The *eta* gene (exfoliative toxin) gene was present in 3 isolates (Table 3).

Correlation of biofilm, antimicrobial resistance and virulence genes

Only 1 of the biofilm producers was found to be resistant to most antibiotics (Table 2). All 5 isolates producing a biofilm showed also haemolytic activity whereas only 1 produced staphyloxanthin (Table 3). The 2 isolates carrying the Panton-Valentine leukocidin (PVL) *lukFS-PV* gene produced both intermediate biofilm. The *sdrD* gene was present in all and the *cna* gene in 4/5 of isolates producing a biofilm (Table 3).

Spa-typing

In total, 22 different *spa*-types and 1 novel sequence repeat (Sequence repeat: 11-19-12-21-10-34-24-24-34-22-25; Kreiswirth ID: YHGFC2BQQBLO) were identified (Table 3). Only 3 isolates belonged to the same *spa*-type t688, one each from a European, African and Asian carrier (Table 3). Two isolates belonged to t437 and were collected from Asia.

Multi-locus sequence types (MLST)

MLST Sequence Types (ST) were determined for all 26 isolates. Sixteen different ST types were identified (Table 3), with the main types being ST5 (n=4), ST8 (n=3) and ST59 (n=3). All isolates within ST5 belonged to *agr* type II, whilst all ST8 and ST59 isolates were *agr* type I (Table 3).

Whole genome sequence analysis of isolate MRSA-3

MRSA-3 was isolated from a veterinary surgeon from South America and was submitted for whole genome sequencing as it had a wide range of antibiotic resistance and was the only isolate to be rifampicin resistant (Table 2). *Spa*-typing and MLST revealed that it belonged to the ST8-t064 clonal lineage. The ClonalFrame phylogenetic tree based on 53 *S. aureus* rMLST genes in Figure 1, shows the evolutionary relationship between MRSA-3 and 181 published *S. aureus* genomes. MRSA-3 was in the same cluster as other ST8 isolates. Further, the ACME locus genes *speG*, *aliD* and *arcA* and the SCC*mec* complex genes, *mecA*, *mecR*, *ccrA*, *ccrB* and *orfX* were all present in MRSA-3. The phylogenetic relationship of MRSA-3 to the other MRSA genomes is shown for the *mecA* gene on the neighbour joining tree (Figure 2a; MRSA-3 indicated by black arrow and dark red filled square).

The MRSA-3 isolate was resistant to rifampicin (MIC ≥32 mg/L). Sequence NCBI BLAST analysis against the reference sequence of rifampicin susceptible *S. aureus* strain ATCC 25923 (GenBank accession number CP009361) (MIC, ≤0.5 mg/L) revealed 20 nucleotide changes in the 3552 bp *rpoB* sequence. However, only 3 nucleotide changes at position 1411 (GGA →AAC), 1430 (GCT →GAT) and 2210 (TTT →TAT) resulted in amino acid changes 471 (D→N; Asp→Asn), 477 (A→D; Ala → Asp) and 737 (F→Y; Phe → Tyr), respectively (Figure 3). The changes in MRSA-3 were not observed in the other published *S. aureus* ST8 genomes (Figure 2b).

Discussion

In this study, 26 nasal MRSA isolates obtained from human and veterinary surgeons were phenotypically and genotypically characterised. These 26 MRSA isolates were collected from a large international cohort of 1,166 human and 60 veterinary surgeons from Africa, Asia, Central America, Europe and South America. As previously described, the MRSA colonization rate amongst the human surgeons was 2% (23/1,166; 95% CI 1.3;2.9) indicating a colonization rate equivalent to the general population and from HCWs (5, 6, 9, 28-30). The MRSA rate for the

297 veterinary surgeons was 5% (3/60; 95% CI 1.0;13.9) which lies also in the colonization rate
298 reported for veterinary personnel (31).
299
300 The most prevalent STs in this study were ST5 and ST8. The 4 ST5 isolates were collected (one
301 each) from a European, an African, an Asian and from a Central American surgeon. The ST5
302 lineage is associated with the successful global HA-MRSA New York/Japan clone (ST5,
303 USA100) and the "paediatric" clone (ST5) both belonging to one of the major MRSA clonal
304 complexes CC5 (12, 32). However, polyclonal genesis within this lineage has been shown (12, 32)
305 and the only consistencies within the 4 ST5 isolates in this study were that they all belonged to *agr*
306 type II, and were negative for the genes *lukFS-PV, tst* and *eta*.
307 In total, 3 isolates (2 from Asian surgeons and 1 from a South American veterinary orthopaedic
308 surgeon) belonged to the ST8 (USA300) clonal lineage. This lineage is mainly associated in the
309 US as CA-MRSA responsible for the most frequent and severe skin and soft tissue infections in
310 emergency departments (11, 33). The reason for this is believed to be due to higher virulence
311 including the production of toxins (12). This is consistent with the expression of staphyloxanthin
312 and haemolytic activity in all 3 ST8 isolates as well as the possession of the *tst* gene in the 2 Asian
313 isolates and the *eta* gene in the South American isolate.
314 In most cases, the described ST-*spa*-type combinations identified in this study have previously
315 been associated with increased severity and patient mortality in hospital settings. For example the
316 ST1-t386 lineage from 1 Asian surgeon belonged to CC1 and is mainly associated with USA400
317 ORSA IV, a CA-MRSA in the USA that caused paediatric deaths in the Midwest of the United
318 States (11). Furthermore, the 2 ST59-t437 isolates from Asian surgeons belonged to a well
319 described CA-MRSA clonal lineage in Asia causing skin and soft tissue infections as well as
320 sepsis and severe pneumonia (34, 35). A recent study has also identified ST59-t437 isolates in
321 several European countries demonstrating that *S. aureus* lineages are not restricted to particular

geographical regions or specific host environments (36), although in our study all ST59 isolate were associated with Asian surgeons.

The importance of antibiotic use in food production and the risks of emergence of multiply antibiotic resistance pathogens within the food chain has resulted in an increasing awareness of the risk of transmission of MRSA within the sector, including veterinary orthopaedic surgeons (13-15). In our study, 3 veterinary orthopaedic surgeons were culture positive for MRSA (2 from Europe, 1 from South America). Two of them were colonized with LA-MRSA clonal lineages, i.e. lineages previously cultured directly from livestock. For example, 1 veterinary orthopaedic surgeon from Europe was colonized with MRSA ST398-t011, belonging to CC398. CC398 is the most predominant LA-MRSA clonal lineage reported across the world (13-15), and has been detected in a variety of domesticated animals (13-15). CC398 has often been reported to be transmitted from animals to humans (13-15) with most human CC398 infections being superficial skin and soft tissue infections (14). The second veterinary-associated MRSA (from a South American veterinary surgeon) was also carrying a known clonal lineage, ST8-t064 (37).

Furthermore, this ST8-t064 (MRSA-3) isolate was multiply antibiotic resistant including resistance to rifampicin. Resistance to rifampicin is a significant challenge in the treatment of orthopaedic device related infection since rifampicin is the sole clinically available antibiotic with significant activity against staphylococcal biofilms (38, 39). Whole genome sequencing revealed 3 SNPs in the *rpoB* sequence, which led to a change in the amino acid sequence at positions 471, 477 and 737. The amino acid change at position 477 from Alanine to Aspartic acid has been shown to be responsible for high level resistance to rifampicin (40-42).

Comparison of MRSA-3 SCC*mec* complex genes, (*mecA, mecR, ccrA* and *ccrB)* and *orfX* gene to the other ST8 *S. aureus* genomes from NCBI revealed a close evolutionary relationship. In contrast, the *rpoB* neighbour joining tree showed that the relationship between MRSA-3 and the other ST8 isolates is more diverse, indicating that it has a different evolutionary history. The

ACME locus genes: *aliD, arcA* and *speG* were present in the MRSA-3 indicative for most ST8 (USA300) CA-MRSA (43) while the *lukFS-PV* gene encoding PVL was absent.

The phenotypic evaluation of all MRSA isolates revealed that the isolates displayed a range of staphyloxanthin production, haemolytic activity and biofilm forming potential. Screening for the most prevalent MSCRAMMs demonstrated the presence of most of the tested virulence genes in the majority of the 26 MRSA isolates. For example, the surface protein clumping factor B (*clfB*) present in all 26 surgeons isolates has been shown to promote attachment of *S. aureus* to human squamous nasal epithelial cells by binding to keratin-10 (1). In total 34.6% of the nasal MRSA either carried the PVL *lukFS-PV* gene, responsible for increased severe disease and clinical symptoms, including necrotic lesions of the skin (10, 11), the *eta* gene coding for exfoliative toxin enhancing the transmission of MRSA through skin-skin contact, due to the destruction of the epidermal barrier (44) or the toxic shock syndrome *tst* gene. This highlights the highly virulent potential of these nasal colonizing MRSA isolates.

Conclusion

The findings of this study have shown that the MRSA isolates cultured from surgeons possessed genes for a wide range of virulence factors and toxins but also belonged to clonal lineages described for their high transmission potential and are associated with increased infection severity and mortality. This indicates not only that the surgeons are a potential risk in spreading these lineages in clinical and healthcare settings, but also that they themselves are exposed to the acquisition of such isolates.

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Legends

Figure 1. Population structure of MRSA-3 (indicated by a black arrow and a dark red filled square) and 181 published *S. aureus* isolates constructed from 53 rMLST genes and implemented in ClonalFrame. Isolates labelled according to ST complex: ST1 (turquoise); ST5 (pink); ST8 (red); ST22 (light blue); ST30 (blue); ST45 (green); ST398 (orange); singletons (yellow). The scale (0.1) is in coalescent units and represents the number of substitutions per site.

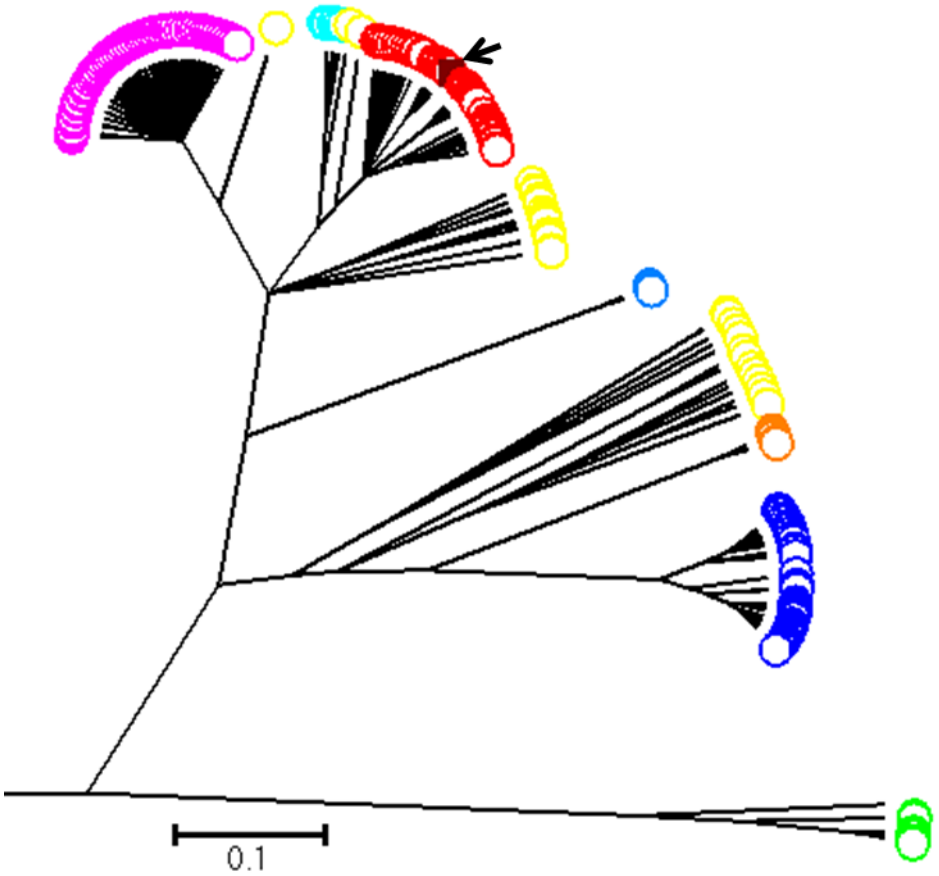

Figure 2. Phylogenetic trees of MRSA-3 (indicated by a black arrow and a dark red filled square) and 181 published *S. aureus* genomes based on the presence of: (a) *mecA* and (b) *rpoB*. Isolates labelled according to ST complex: ST1 (turquoise); ST5 (pink); ST8 (red); ST22 (light blue); ST30 (blue); ST45 (green); ST398 (orange); singletons (yellow). The scale bar (0.001) is in coalescent units and represents the number of substitutions per site.

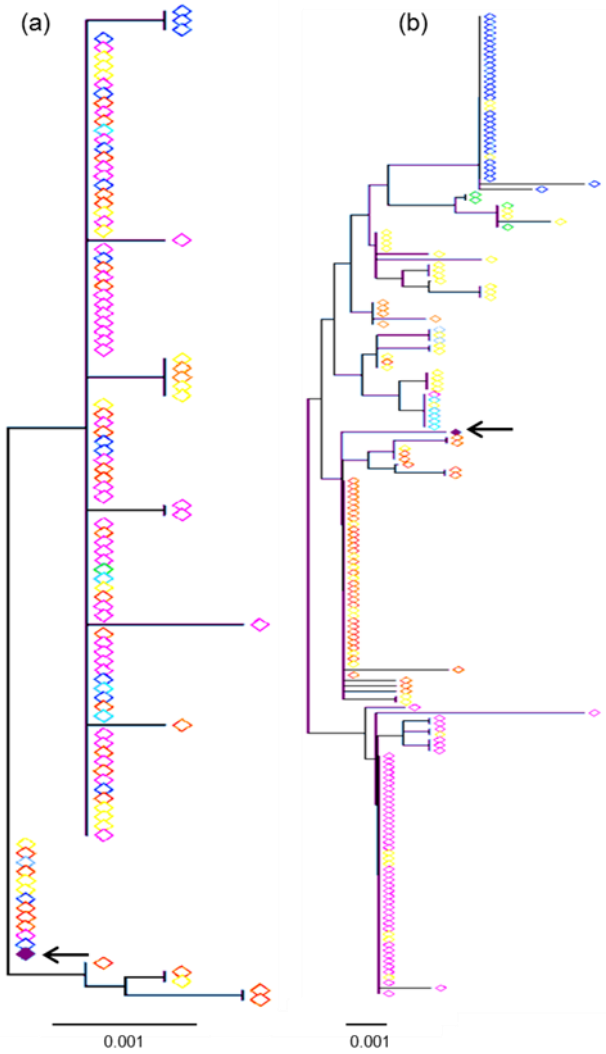

Figure 3. Amino acid alignment of the *rpoB* gene region (421-780) in the rifampicin resistant MRSA-3 and the rifampicin susceptible *S. aureus* ATCC 25923 (GenBank accession number CP009361). White letters on black indicate the 3 amino acid changes identified in MRSA-3.

```
MRSA-3          IGLSRMERVVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQFMNQANPLDELT   480
ATCC 25923      IGLSRMERVVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQFMDQANPLAELT

MRSA-3          HKRRLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIETPEGPNIGLINSLSSYARVNEFG   540
ATCC 25923      HKRRLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIETPEGPNIGLINSLSSYARVNEFG

MRSA-3          FIETPYRKVDLDTHAITDQIDYLTADEEDSYVVAQANSKLDENGRFMDDEVVCRFRGNNT   600
ATCC 25923      FIETPYRKVDLDTHAITDQIDYLTADEEDSYVVAQANSKLDENGRFMDDEVVCRFRGNNT

MRSA-3          VMAKEKMDYMDVSPKQVVSAATACIPFLENDDSNRALMGANMQRQAVPLMNPEAPFVGTG   660
ATCC 25923      VMAKEKMDYMDVSPKQVVSAATACIPFLENDDSNRALMGANMQRQAVPLMNPEAPFVGTG

MRSA-3          MEHVAARDSGAAITAKHRGRVEHVESNEILVRRLVEENGVEHEGELDRYPLAKFKRSNSG   720
ATCC 25923      MEHVAARDSGAAITAKHRGRVEHVESNEILVRRLVEENGVEHEGELDRYPLAKFKRSNSG

MRSA-3          TCYNQRPIVAVGDVVEYNEILADGPSMELGEMALGRNVVVGFMTWDGYNYEDAVIMSERL   780
ATCC 25923      TCYNQRPIVAVGDVVEFNEILADGPSMELGEMALGRNVVVGFMTWDGYNYEDAVIMSERL
```

557 Table 1. Primers used in the molecular identification of virulence factors in this study[1]

Gene	Primer name	Sequence (5`- 3`)	Product size (bp)	Annealing temp (°C)
spa-typing				
spa	1095F	AGACGATCCTTCGGTGAGC		60
	1517R	GCTTTTGCAATGTCATTTACTG		
agr-typing				
agr	agr1-4-1	ATGCACATGGTGCWCATGC		
	agr1-2	GTCACAAGTACTATAAGCTGCGAT	439	55
	agr2-2	TATTACTAATTGAAAAGTGCCATAGC	572	55
	agr3-2	GTAATGTAATAGCTTGTATAATAATACCCAG	321	55
	agr4-2	CGATAATGCCGTAATACCCG	657	55
MSCRAMMs				
cna	CNA-1	GTCAAGCAGTTATTAACACCAGAC	423	55
	CNA-2	AATCAGTAATTGCACTTTGTCCACTG		
eno	ENO-1	ACGTGCAGCAGCTGACT	302	55
	ENO-2	CAACAGCATYCTTCAGTACCTTC		
ebpS	EBP-1	CATCCAGAACCAATCGAAGAC	186	55
	EBP-2	CTTAACAGTTACATCATCATGTTTATCTTTG		
fnbB	FNBB-1	GTAACAGCTAATGGTCGAATTGATACT	524	55
	FNBB-2	CAAGTTCGATAGGAGTACTATGTTC		
fib	FIB-1	CTACAACTACAATTGCCGTCAACAG	404	55
	FIB-2	GCTCTTGTAAGACCATTTTCTTCAC		
clfA	CLFA-1	ATTGGCGTGGCTTCAGTGCT	292	55
	CLFA-2	CGTTTCTTCCGTAGTTGCATTTG		
clfB	CLFB-1	ACATCAGTAATAGTAGGGGGCAAC	205	55
	CLFB-2	TTCGCACTGTTTGTGTTTGCAC		
sdrC	sdrC-F	ACGACTATTAAACCAAGAAC	560	45
	sdrC-R	GTACTTGAAATAAGCGGTTG		
sdrD	sdrD-F	GGAAATAAAGTTGAAGTTTC	500	45
	sdrD-R	ACTTTGTCATCAACTGTAAT		
sdrE	sdrE-F	CAGTAAATGTGTCAAAAGA	767	45
	sdrE-R	TTGACTACCAGCTATATC		
bbp	BBP-1	AACTACATCTAGTACTCAACAACAG	575	55
	BBP-2	ATGTGCTTGAATAACACCATCATCT		
icaA	icaA-F	GATTATGTAATGTGCTTGGA	770	50
	icaA-R	ACTACTGCTGCGTTAATAAT		
Toxins				
PVL	luk-PV-1	ATCATTAGGTAAAATGTCTGGACA TGATCCA	433	55
	luk-PV-2	GCATCAASTGTATTGGATAGCAAA AGC		
hlg	hlg-1	GCCAATCCGTTATTAGAAAATGC	937	55
	hlg-2	CCATAGACGTAGCAACGGAT		
sea	GSEAR-1	GGTTATCAATGTGCGGGTGG	102	57
	GSEAR-2	CGGCACTTTTTTCTCTTCGG		
seb	GSEBR-1	GTATGGTGGTGTAACTGAGC	164	57
	GSEBR-2	CCAAATAGTGACGAGTTAGG		
sec	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	451	57
	GSECR-2	CACACTTTTAGAATCAACCG		
sed	GSEDR-1	CCAATAATAGGAGAAAATAAAAG	278	57
	GSEDR-2	ATTGGTATTTTTTTTCGTTC		
see	GSEER-1	AGGTTTTTTCACAGGTCATCC	209	57
	GSEER-2	CTTTTTTTTCTTCGGTCAATC		
eta	GETAR-1	GCAGGTGTTGATTTAGCATT	93	57
	GETAR-2	AGATGTCCCTATTTTTGCTG		
etb	GETBR-1	ACAAGCAAAAGAATACAGCG	226	57
	GETBR-2	GTTTTTGGCTGCTTCTCTTG		
tst	GTSSTR-1	ACCCCTGTTCCCTTATCATC	326	57
	GTSSTR-2	TTTTCAGTATTTGTAACGCC		

Methicillin resistance *mecA*	MECA P4	TCCAGATTACAACTTCACCAGG	162	53
	MECA P7	CCACTTCATATCTTGTAACG		

[1]Table was taken and modified from Post et al. (Post et al., 2014)

Table 2. Antibiotic resistance profiles of the MRSA isolates.

Region	MRSA No.[1]	Gentamicin	Tobramycin	Amikacin	Netilmicin	Tetracycline	Erythromycin	Clindamycin	Fusidic acid	Ciprofloxacin	Ofloxacin	Moxifloxacin	Levofloxacin	Trim/Sulf[2]	Rifampicin	Mupirocin
Africa	11	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S
	24[4]	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Asia	7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	19	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S
	22	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S
	8	R	R	R	R	S	S	S	S	R	ND	S	R	R	S	S
	17[4]	S	S	S	S	S	R	R	S	R	R	R	R	S	S	S
	26[4]	R	R	R	R	S	R	R	S	R	R	R	R	S	S	S
	4	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
	16	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S
	1	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S
	12	S	S	S	S	R	R	R	S	R	ND	S	R	S	S	S
	6	R	R	R	R	R	R	R	S	R	R	R	R	S	S	S
	23	S	S	S	S	S	R	R	S	R	R	R	R	S	S	S
	18	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S
	25	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S
Central America	5	S	S	S	S	S	R	R	S	R	R	R	R	S	S	S
Europe	2	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
	13*	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S
	15	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	9*	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
	21	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	20[5]	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
South America[3]	3*	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S
	10[4]	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	14	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
Total (n=26)		8	8	8	8	7	13	13	1	8	6	6	8	3	1	0

[1]All isolates were resistant (R) to: Penicillin, Oxacillin, Piperacillin, Mezlocillin, Ampicillin-Sulbactam, Cefotaxime, Cefuroxime, Ticarcclavulans, Cefoxitin and all isolates were susceptible (S) to: Tigecycline, Fosfomycin, Daptomycin, Vancomycin, Linezolid. ND stands for not determined.

[2]Trim/Sulf: Trimethoprim-Sulfamethoxazole.

[3]MRSA-3 isolate submitted for whole genome sequencing.

[4]Indicates intermediate biofilm production.

[5]Indicates strong biofilm production.

*Indicates MRSA isolated from veterinary surgeons.

Table 3. Summary of the phenotypic and genotypic characterization of the MRSA isolates.

Region	Isolate No.	MLST	CC[1]	*spa*-type	*agr*-type[2]	HLG[3]	STX[4]	*lukFS-PV*	*tst*	*cna*	*fnbB*	*ebpS*	*fib*	*s*
Asia	1	ST1	1	t386	III	-	+	-	-	+	-	+	+	-
Africa	11	ST5	5	t688	II	+	-	-	-	-	-	+	+	-
Asia	12	ST5	5	t688	II	+	+	-	-	-	-	+	+	-
Central America	5	ST5	5	t895	II	-	-	-	-	-	-	+	+	-
Europe	9*	ST5	5	t1340	II	+	+	-	-	-	-	+	+	-
Asia	4	ST8	8	t1767	I	+	+	-	+	-	+	-	+	-
Asia	16	ST8	8	New[5]	I	+	+	-	+	-	+	-	+	-
South America	3*	ST8	8	t064	I	+	+	-	-	-	+	-	+	-
Asia	6	ST45	45	t14861	0	+	-	-	-	+	-	+	-	-
Europe	15	ST45	45	t230	I	-	-	-	-	+	-	+	-	-
Asia	19	ST59	59	t437	I	+	-	-	-	-	-	+	+	-
Asia	25	ST59	59	t437	I	+	+	-	+	-	-	-	+	-
Asia	22	ST59	59	t441	I	+	+	-	+	-	-	-	+	-
Asia	8	ST72	8	t3092	I	+	-	-	-	-	-	+	+	-
Asia	18	ST72	8	t324	I	+	-	-	-	-	-	+	+	-
Africa	24[#]	ST88	88	t786	III	+	+	-	-	-	+	-	+	-
Asia	7	ST97	97	t267	I	+	+	-	-	-	+	+	+	-
South America	10[#]	ST207	S	t525	III	+	-	-	-	+	+	+	+	-
Asia	17[#]	ST217	S	t852	I	+	-	+	-	+	-	-	-	-
Europe	13*	ST398	398	t011	0	-	-	-	-	+	+	-	-	-
Europe	20[$]	ST425	S	t6292	II	+	-	-	-	+	-	+	+	-
Europe	21	ST508	S	t1203	I	-	-	-	-	+	-	+	-	-
South America	14	ST641	S	t002	II	-	-	-	-	-	-	+	+	-
Europe	2	ST2112	S	t688	II	-	+	-	-	-	-	+	+	-
Asia	26[#]	ST2124	S	t7428	I	+	-	+	-	+	-	-	-	-
Asia	23	ST2124	S	t9446	I	-	-	-	-	+	-	-	-	-

[1] CC: Clonal complex with S: S: singeltons.
[2] *agr*-type 0: no PCR product for any of the 4 *agr* types was obtained.
[3] HLG: Haemolytic activity, (+ /- indicates active / not active).
[4] STX: Staphyloxanthin production (+ /- indicates production / no production).
[5] Kreiswirth ID: YHGFC2BQQBLO.
[#] Indicates intermediate biofilm production.
[$] Indicates strong biofilm production.

NB: + / - indicates a positive / negative PCR amplification *indicates the veterinarian nasal MRSA isolates. The *hlg*C/B, *i*
present in all isolates. Ninety-six percent of isolates carried the *sdrE* gene.